THE DEVELOPMENT OF *XENOPUS GILLI* ROSE & HEWITT (ANURA, PIPIDAE)

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(With 10 figures)

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ABSTRACT

Tadpoles of the Cape clawed frog *Xenopus gilli* can be distinguished from those of *Xenopus laevis* by the distribution of melanophores and differences in dimensions. *Xenopus gilli* breeds in cooler water and is less tolerant of rising water temperatures. The species seems to be more abundant in mountainous terrain than in the low-lying Cape Flats. The breeding period largely coincides with that of *Xenopus laevis*, but indications are that it starts and ends earlier in the year. Where both species occur together some specimens appear to be hybrids.

CONTENTS

INTRODUCTION

The Cape clawed frog, as it should preferably be called (Mertens 1970), was recognized as a new species and described as *Xenopus gilli* by Rose & Hewitt in 1927. The type locality of this pretty and easily distinguishable species is the Silvermine Stream near Clovelly, Cape Peninsula. A smaller, more pointed head, the absence of the subocular tentacle and the distinctive coloration render it quite different from the more common *Xenopus laevis*, which reaches a much greater maximum size. Both species often occur together, and the validity of the species *X. gilli* has sometimes been questioned.

Recently *X. gilli* has been confirmed as a true species. H. Kobel (*in litt.* 1977) of the Zoology Department of the University at Geneva succeeded in breeding *Xenopus gilli*, and also in cross-breeding *X. gilli* with *X. laevis*. The male hybrids proved to be sterile, while the female crosses could reproduce when mated with either *X. gilli*, *laevis* or *muelleri* (see Kobel & Du Pasquier 1975; Wabl & Du Pasquier 1976).

In the sandy Cape Flats only a few metres above sea-level, one specimen of

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Xenopus gilli is occasionally found amongst approximately 1 000 *X. laevis* which are being caught commercially for export as laboratory animals (J. Wood pers. comm.). Sometimes, during the dry season, several specimens of *X. gilli* have been found hibernating together under logs, etc., or encapsulated into the mud of dried-up vleis in the Cape Flats.

There are two specimens, male and female, in the collection of the South African Museum (ZR18914), which were collected at Citrusdal, in approximately 1937. Several *Xenopus* specimens, originally considered to be *X. laevis*, were reidentified after the establishment of *X. gilli* as belonging to that species (Mertens 1970), as well as a male specimen at the South African Museum (ZR2346) collected at Willemsrivier (31°21′S 19°06′E) west of Calvinia in 1898.

Both Citrusdal and Willemsrivier are within the winter-rainfall area of the south-western Cape. These localities have not been reconfirmed, however, nor has *Xenopus gilli* been recorded between the Cape Flats and the already-mentioned northern localities. The reason might be that a thorough search for this species has not yet been conducted in the area. In addition, *Xenopus gilli* is usually not easily found as the frogs hide in the mud or leaf-layer at the bottom of the pond. Occasional migration over land of this aquatic species, as is known to happen with *Xenopus laevis*, probably does occur. This could perhaps explain why in a given locality *Xenopus gilli* is found in one year and not in the following years.

It is also possible that the two northern localities represent isolated occurrences, especially if *Xenopus gilli* is a relict of a formerly more widely distributed form. *Xenopus laevis* lives in both acidic and alkaline waters (Nieuwkoop & Faber 1956) ranging from clear, cold, fast-running mountain streams to shallow, warm, muddy vleis. If, indeed, the essentially tropical *Xenopus laevis*, which occurs in most of eastern and southern Africa from the Red Sea to the western Cape (Mertens 1970), has invaded the range of the winter-rainfall species *Xenopus gilli*, the obviously greater tolerance to environmental changes of *X. laevis* might well be detrimental to the more specialized *Xenopus gilli*.

A clarification of the present range of *Xenopus gilli* and the question of possible competition with *Xenopus laevis*, especially in view of possible protective measures, is desirable. As described above, the presence of *Xenopus gilli* is not easy to establish when depending, as hitherto, on the fully developed frog alone. This suggested the use of the larval stages for distribution investigation. While the development of *Xenopus laevis* is fully documented (Nieuwkoop & Faber 1956), both eggs and larvae of *Xenopus gilli* remained unknown (Wager 1965).

Investigations during the past few years revealed that in several ponds and dams within the plateau of the mountainous Cape Point Nature Reserve *Xenopus gilli* is comparatively common. This in all probability is due to the curious fact that *Xenopus laevis* is comparatively rare in these localities.

To establish whether *X. gilli* can be identified in its larval stages and also to prove or disprove the species validity through cross-breading with *Xenopus*

laevis, breeding experiments were undertaken by the author during 1976 at the South African Museum and by S. McVeigh at the Fauna and Flora Section of the Cape Department of Nature and Environmental Conservation. While the females responded to the hormone treatment by laying eggs, the males showed no reaction. One female, 5,3 cm in length (snout to vent), laid approximately 270 eggs within a few hours with a yolk-size of 1,4–1,8 mm. Thus the larval stages still remained undescribed.

MATERIALS AND METHOD

The natural breeding season of *Xenopus laevis* is given as September to December for the Stellenbosch–Cape Town area (Nieuwkoop & Faber 1956). It was thought that, since *Xenopus gilli* is a true western Cape winter-rainfall species, its breeding season could well start as early as winter. *Xenopus* tadpoles were caught during metamorphosis in January–February 1976 in the Cape Point Reserve. They completed development in an aquarium and proved to be *Xenopus gilli*. This, and the dominance of *Xenopus gilli* over *Xenopus laevis* in the reserve, led to the checking of various water accumulations in the reserve on 31 July 1977. *Xenopus* larvae, approximately 12–15 mm in length, were found in coffee-coloured water with a pH of 5, and with a temperature of 12°C at 11h00 at a depth of 25 cm.

Some of these larvae were reared in an aquarium in the open, and the ponds in the Cape Point Reserve were checked periodically for new spawnings and to record the development of the tadpoles in natural conditions. Several tadpoles of various stages were kept in a plastic gauze cage 1 m × 0,5 m × 0,5 m, which was fixed between poles at the edge of a pond, leaving the upper edge 10 cm above the water. This facilitated observations on the development of specific, free-living individuals. In addition, approximately 100 preserved larvae and young frogs of *Xenopus gilli*, collected in the same area and fixed in Lenhossek fluid or alcohol, were used for the study.

For comparison the following material of *Xenopus laevis* was examined: preserved larvae and young frogs collected by N. A. H. Millard in the Cape Flats during the 1940s and by the author in the Cape Flats during 1977; a series of stage-determined larvae collected by Hubrecht Laboratory, Utrecht, Holland, at Stellenbosch in 1949–50 and housed in the South African Museum; and live larvae reared in an aquarium during 1977.

In the following description, which is the object of this paper, the stage numbers refer to those of the Normal Table of *Xenopus laevis* laid down by Nieuwkoop & Faber (1956).

DEVELOPMENT

While there are changes in body-proportions and pigment-distribution throughout development, free-swimming larvae of *Xenopus gilli* can be distinguished from those of *Xenopus laevis* by their unpigmented longitudinal

bands on the dorsal surface of the head (Fig. 1A). These occur, one on either side, a short distance lateral to the central nervous system (seen by transparency). The bands embrace the eyes from where they run in an S-shape inwards then backwards towards the trunk. The two bands together form a lyre shape, which, even in the water, is very conspicuous, the rest of the body being very dark. From approximately Stage 45 onwards isolated melanophores begin to appear

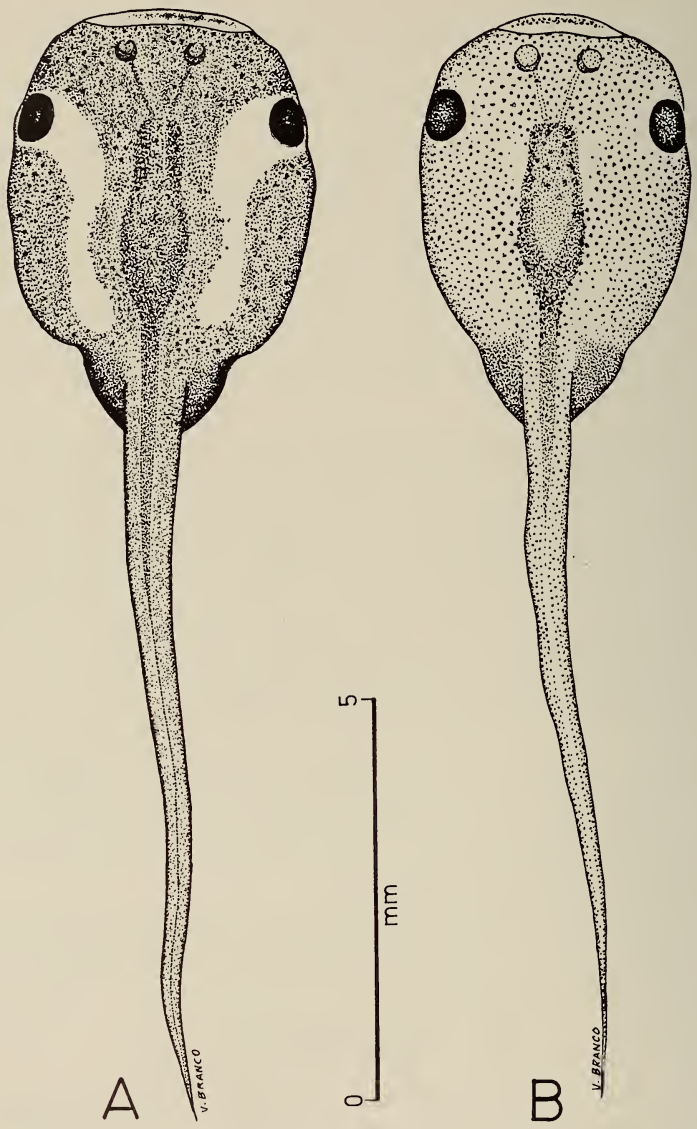

Fig. 1. A. *Xenopus gilli* larva, Stage 45–46, dorsal. B. *Xenopus laevis* larva, Stage 45–46, dorsal.

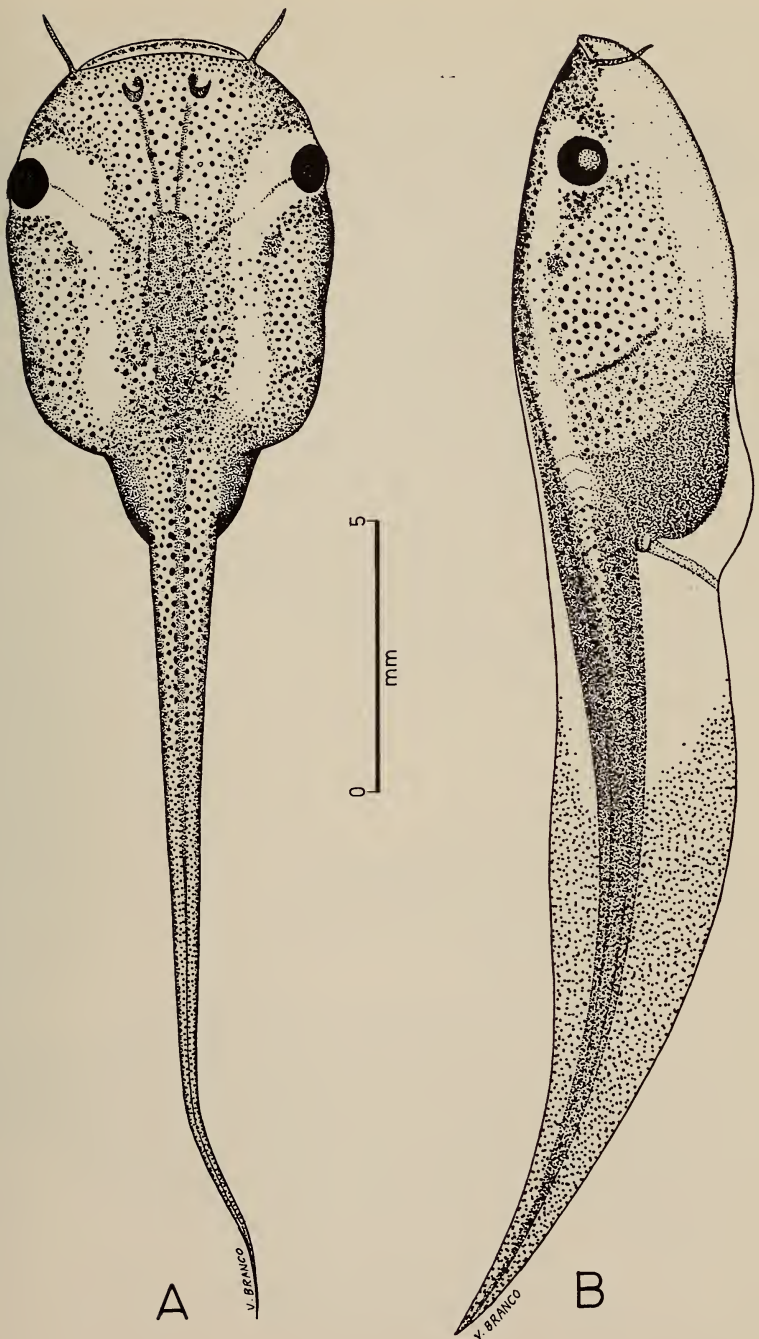

Fig. 2. *Xenopus gilli* larva, Stage 51. A. Dorsal. B. Lateral.

Fig. 3. Photographs of heads of live larvae, Stage 51, dorsal. A. *Xenopus gilli*. B. *X. laevis*.

in the lyre in the thymus gland region. They increase in number during further development, but never seem to reach the density of the centre portion and the sides of the head (Figs 2, 3A). The posterior third of the lyre remains unpigmented until at least Stage 56, while the areas surrounding the eyes can become somewhat pigmented from Stage 55 onwards (Fig. 5). However, in most specimens the lyre, although partly obscured, remains distinguishable well into metamorphosis (Fig. 5).

In contrast, *Xenopus laevis* larvae from an early stage usually have a more or less uniform distribution of pigment on the dorsal surface of the head (Fig. 1B), except sometimes in a position corresponding to the last portion of the lyre, which may be unpigmented. However, in very young *Xenopus laevis* larvae (free-swimming, but before Stage 44) there may be some unpigmented areas on either side of the central nervous system, which remind one of the lyre of *X. gilli*, though the anterior part is quite different (Fig. 6). This condition was found in ten out of twenty specimens of the same batch and size. But even in these specimens the posterior portion of the 'lyre' usually becomes pigmented very early and is often indistinct at Stage 46, when the areas between eyes and central nervous system have usually also become pigmented (Fig. 1B). In the water the only dark portions which show up are the central nervous system, the eyes and the abdomen.

The thymus gland, which is situated between eye and ear just below the surface, and which becomes pigmented in both species at Stage 49, is more conspicuous in *Xenopus laevis*, appearing almost like a second pair of eyes (Figs 3, 4). In *Xenopus gilli*, this gland is situated just outside the lyre and is somewhat obscured by the heavy pigmentation in the layers above it.

The pigmentation in the dorsal and ventral tail-fins starts at the hind end and increases in an anterior direction. In *Xenopus gilli* the pigmentation of the ventral fin extends as a narrow band along the edge, and reaches the cloaca at Stages 55–56 (Fig. 5), while in *Xenopus laevis* the only fin pigmentation near the cloaca is an isolated patch which appears at Stage 56 (Nieuwkoop & Faber 1956).

While melanophores appear on the hind-limb buds of *Xenopus laevis* at Stage 51, in *Xenopus gilli* the hind-limb buds are already pigmented at Stage 48. At the same stage in *X. gilli*, and sometimes earlier, a whitish spot becomes visible above the anterior portion of the central nervous system. In later stages the 'Stirnorgan', in the form of a spherical opaque structure which pushes up the skin above it, can be observed in this spot (Fig. 5). In *Xenopus laevis* an unpigmented spot is said to appear above the 'Stirnorgan' at Stage 57. The 'Stirnorgan' is developed from the pineal body and lies between the epidermis and the skull (Nieuwkoop & Faber 1956).

The naso-lachrymal duct is indicated in *Xenopus gilli* tadpoles at Stages 55–56 when the pigment begins to part along a line roughly parallel to the upper lip, from the outer corner of the nostril to where it meets a triangular, unpigmented area in front of the eye (Fig. 5). This unpigmented line is very

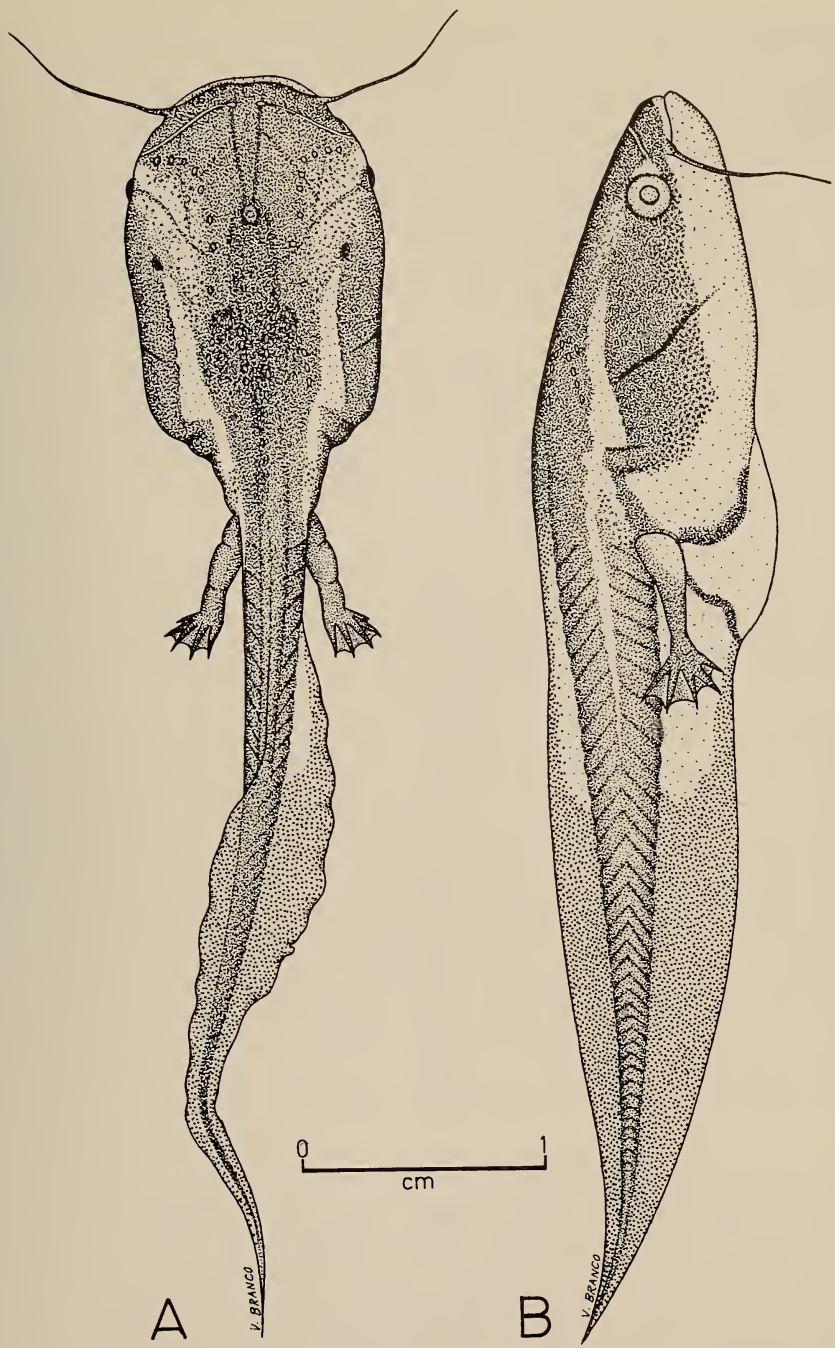

Fig. 5. *Xenopus gilli* larva, Stage 57. A. Dorsal. B. Lateral.

clear and narrower than the olfactory nerve at Stages 57 and 58, after which melanophores shift over it again. At Stage 60 it is nearly invisible. A somewhat irregular protuberance appears at Stage 61 at the point where the nasolachrymal duct meets the pre-orbital triangle on the lateral wall of the head. The protuberance shifts in a ventro-caudal direction, coming closer to the eye, and reaches its final position ventral to the eye, almost in line with the circumorbital lateral line sensory organs, at Stage 64 (Fig. 7). Two depressions appear on it which at the end of metamorphosis have become oval apertures, facing in a slightly caudal direction. Due to its size and position this raised area bearing the

Fig. 6. *Xenopus laevis* larva, pre Stage 44, dorsal.

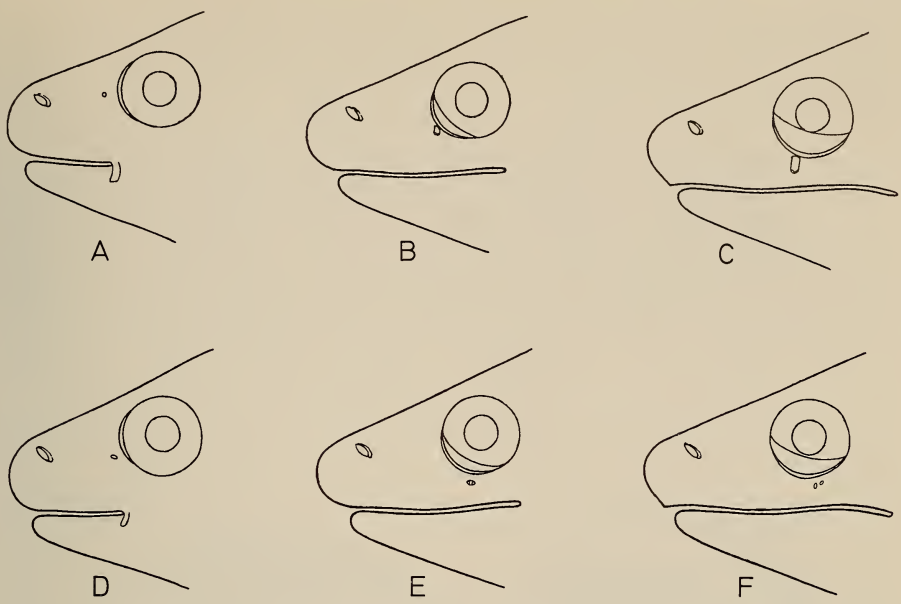

Fig. 7. Development of nictitating membrane and external aperture of nasolachrymal duct, Stages 62, 64, 66 (metamorphosis completed). A–C. *Xenopus laevis*. D–F. *Xenopus gilli*.

external openings of the nasolachrymal duct can easily be mistaken for one of the sensory organs. However, in adult stages the protuberance is usually flattened out and only two openings remain.

Föske (1934 from Paterson 1939*b*) believed that the nasolachrymal duct develops within a very short period from the lower layer of the epidermis. He was, however, unable to find a developmental stage in support of this view. It is likely that the parting of the pigment above the future nasolachrymal duct in *X. gilli*, as described above, supports Föske's view.

The nictitating membrance ('eyelid') forms parallel with this development. In *Xenopus gilli* at Stage 59 the posterior margin of the unpigmented pre-orbital triangle becomes raised and shows a vertical fold close to the eye. The fold lengthens during further development and shifts along the ventral margin of the eye in a caudal direction, absorbs the unpigmented triangle and becomes the nictitating membrane (Fig. 7).

In *Xenopus laevis* the development of the 'eyelid' is similar. The posterior end of the nasolachrymal duct is first visible at Stage 62, when a small, indented semicircular protuberance appears near the anterior tip of the unpigmented pre-orbital triangle (Fig. 7A). At Stage 64 this protuberance is longer than wide, oval in cross-section and has two apertures at its tip, separated by a short septum. It increases in length, comes closer to the eye and, together with the nictitating membrance, shifts ventrad to the eye, where it reaches its final position at Stage 65, forming the subocular tentacle (Fig. 7).

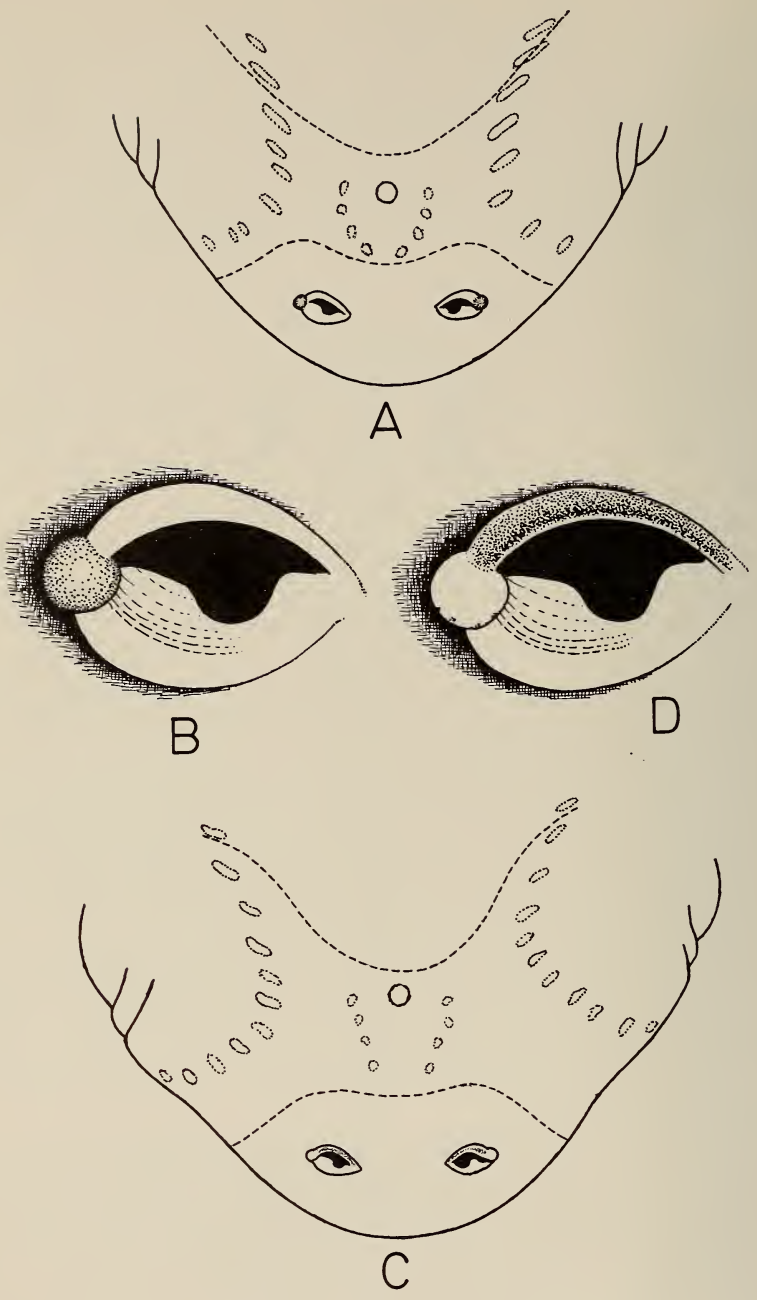

Fig. 8. Head and nostril, Stage 59 onwards, dorsal.
A–B. *Xenopus laevis*. C–D. *Xenopus gilli*.

This so-called subocular tentacle, which is not a true tentacle (Paterson 1939*a*, 1939*b*) sometimes retains both openings in mature frogs. Often, possibly through wear, the short septum disappears so that the two openings unite partially or fully.

Contrary to expectation the nasolachrymal duct in both species has no contact with the orbital capsule. Paterson (1939*b*) concluded that had there been movable eyelids the duct would have been directed inwards towards the eye and its aperture would then have been comparable with the punctum lacrimale of higher forms. Observation of living adult *Xenopus laevis* reveals that the slightly opaque nictitating membrane can move in an upward direction, over the surface of the eye, to its upper margin. This, however, seems to happen only when irritation is felt.

At Stages 55–56 of *X. gilli* the nostril has its outer corner well sunk below the surface of the head. Its caudal margin begins to develop a cone-like protrusion, roughly where the olfactory nerve meets it. This protrusion shifts outwards and becomes situated at the outer corner at Stage 59, where it remains. During these stages the margins of the nostril become raised progressively towards the outer corner, so that at Stage 59 the inner corner is level with the head-surface, while the outer is raised well above it. The lip-like raised margins are now pigmented while the 'knob' is unpigmented (Fig. 8C–D). Soon after the completion of metamorphosis the 'knobs' at the outer corners of the nostrils become pigmented and the darkish belly-dotting appears. At the completion of metamorphosis the frogs measure approximately 15 mm from snout to vent.

This development of nostril and nasolachrymal duct is much the same as in *Xenopus laevis*. However, in the latter the 'knob' is usually pigmented, while the 'lips' are almost unpigmented (Fig. 8A–B).

Thus, while identification of *Xenopus gilli* tadpoles by the lyre becomes less easy in advanced stages, the reversed pigment-pattern of the nostril-margins provides a useful character.

At Stage 60 a light dorsal midline appears on the head and body of *Xenopus gilli*, beginning roughly between the eyes and ending roughly between the hind legs. The typical *gilli*-pattern of more or less complete longitudinal dark bands, two on the back and a weaker, more broken one, on either side, is fully differentiated at Stage 62 (Fig. 9A).

FURTHER OBSERVATIONS ON LIVING LARVAE

The duration of development from the fertilized egg to the end of metamorphosis probably varies. For *Xenopus laevis*, reared in the laboratory, it is given as approximately 58 days (Nieuwkoop & Faber 1956). *Xenopus gilli* larvae examined here were first seen when at Stage 45–46 (probably one week old) on 31 July 1977. On 6 November 1977 approximately 15 per cent of the larvae in the ponds had reached Stage 62. On 20 November some had completed metamorphosis. The development thus required approximately 120 days. This much longer period was probably partly due to the low water temperature, which

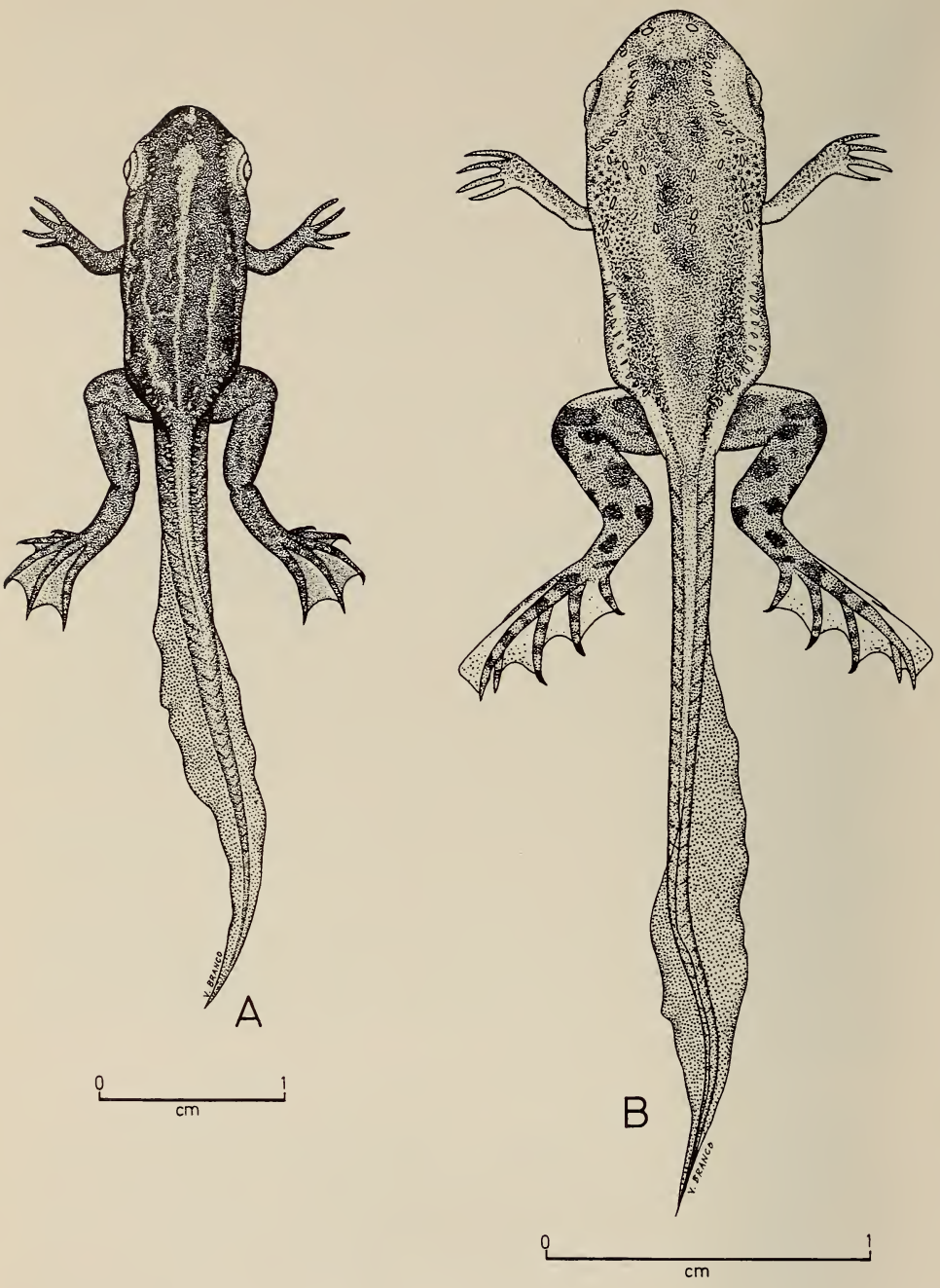

Fig. 9. A. *Xenopus gilli* larva, Stage 62, dorsal.
B. *Xenopus laevis* larva, Stage 62, dorsal.

on 6 November 1977, a warm, sunny day, measured only 20°C at 11h00 in 25 cm depth, whereas Nieuwkoop & Faber retained the water temperatures for their developing larvae at 22–24°C throughout. All larvae observed in three different ponds in the Cape Point Reserve on 31 July and 7 August 1977 were of roughly the same size and stage. During subsequent examinations small and more advanced larvae were encountered together, indicating additional spawning. However, on 6 November the smallest larvae were in Stage 48–49, probably indicating that spawning had ceased. In February 1976 *Xenopus gilli* larvae from Stage 54 to the end of metamorphosis had been collected in the biggest pond where the temperature might have remained sufficiently low. *Xenopus gilli* larvae show signs of discomfort when the water temperature reaches 27°C. They remain at the bottom and try to go even deeper. At 31°C they make sporadic attempts to swim, but balance-disturbance causes them to tumble, often sinking to the bottom, where they remain motionless, sometimes lying on the side or back. In contrast, *Xenopus laevis* larvae still behave normally at a temperature of 34°C. Only when 36°C is reached, do they show signs of dying.

During the night the larvae of both species darken considerably. The tail-fin especially appears practically black when suddenly brought into light.

Larvae reared in an aquarium show retarded growth, although the development seems to progress normally, thus producing smaller frogs. Two *Xenopus gilli*, which were caught near the end of metamorphosis in February 1976 in the Cape Point Reserve, were kept indoors thereafter and did not develop the typical *gilli* belly-dotting and ochrous undersurfaces of the thighs. The nostril 'knob', too, remained unpigmented as in advanced *Xenopus gilli* larvae. In all other respects they showed the *gilli* characteristics and measured approximately 43 mm from snout to vent in November 1977.

Occasionally, when both species occur together, some specimens appear to be hybrids. Their size and general shape is that of *Xenopus laevis*, and so is the presence of the subocular tentacle just ventral to the eye. Both dorsal and ventral coloration is that of *Xenopus gilli*, the longitudinal dark bands being sometimes more complete than in some specimens of *Xenopus gilli*.

A specimen (SAM–ZR44317), collected by B. Deyer in March 1975 in the Lotus River near the entry into Zeekoevlei and donated to the museum by R. Boycott in March 1977, is peculiar in some aspects: its general shape, the lack of a subocular tentacle, the dense pigmentation of the webbing of the feet and the dark belly dotting are typical of *Xenopus gilli*; the teeth in the upper jaw are, as in *Xenopus gilli*, long and protrude well beyond the edge of the mouth; however, the dorsal surface of the animal is of a more or less even, greyish colour with no pattern; the general colour is lighter than is usual in *Xenopus gilli* and the claws on both feet are unpigmented (some unpigmented claws do occur occasionally in both species). The specimen, with a snout-to-vent length of 67 mm, exceeds the maximum size given for *Xenopus gilli* (Poynton 1964).

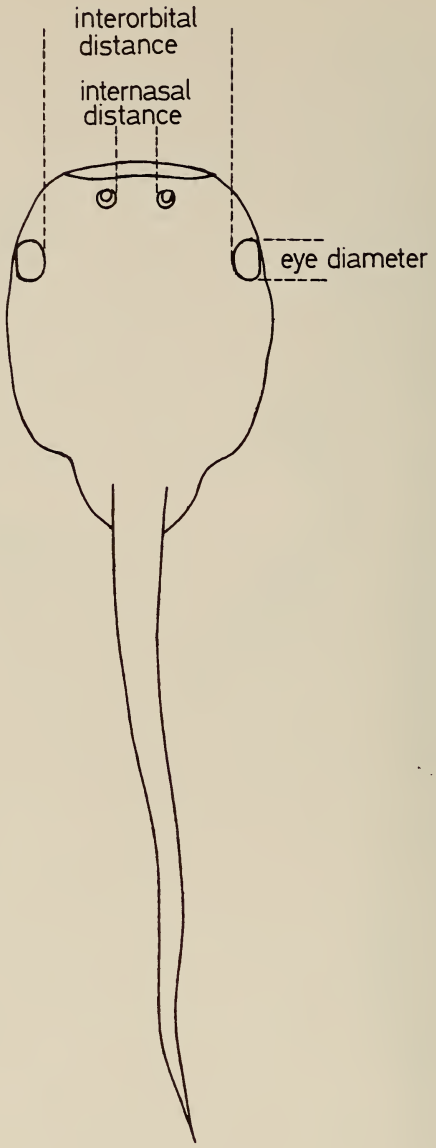

Fig. 10. Diagram to illustrate dimensions used to distinguish *Xenopus gilli* from *Xenopus laevis* larvae.

SUMMARY OF DISTINGUISHING FEATURES OF *X. GILLI* AND *X. LAEVIS* LARVAE

It should be noted that the material on which the dimensions are based consists of freshly preserved specimens in the case of *X. gilli*, and in *X. laevis* of both freshly preserved specimens and specimens which have been preserved for about 30 years.

	X. gilli	*X. laevis*
1.	Tadpoles very dark with unpigmented lyre.	Tadpoles light with more or less even pigmentation.
2.	Thymus gland and blood-vessels on head-body not conspicuous.	Thymus gland and blood-vessels on head-body conspicuous.
3.	Eyes comparatively small; eye diameter/interorbital distance, Stage 45–46: 0,20–0,32 $\bar{x}_{20}$ 0,26; Stage 50–51: 0,18–0,22 $\bar{x}_{15}$ 0,20 (see Fig. 10).	Eyes comparatively large; eye diameter/interorbital distance, Stage 45–46: 0,30–0,54 $\bar{x}_{16}$ 0,34; Stage 50–51: 0,23–0,46 $\bar{x}_{15}$ 0,33.
4.	Nasal capsules comparatively far apart; internasal distance/eye diameter, Stage 45–46: 0,83–1,40 $\bar{x}_{20}$ 1,04; Stage 50–51: 0,62–0,90 $\bar{x}_{15}$ 0,74 (see Fig. 10).	Nasal capsules comparatively close together; internasal distance/eye diameter, Stage 45–46: 0,43–0,88 $\bar{x}_{16}$ 0,63; Stage 50–51: 0,38–0,80 $\bar{x}_{15}$ 0,53.
5.	Nostril 'knob' unpigmented; raised margin of nostril pigmented.	Nostril 'knob' pigmented; raised margin of nostril unpigmented.
6.	Pigmentation of ventral tail-fin reaching cloaca from Stage 55 onwards.	Pigmentation of ventral tail-fin not reaching cloaca but forming an isolated patch near cloaca at Stage 56.

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